combined into one dataset per environment containing all of the spectra collected so far. These datasets were used to construct chemometric regression models to study the effects of time on the Raman spectra of menstrual blood with the PLS Toolbox.

Example 9—Results of Example 8

Ambient Environment

FIG. **18** shows the mean spectra collected in the ambient environment at all 14 time points, after baseline correction, smoothing, and normalization. On average, the intensity of the peak at 1246 $cm^{-1}$, which is assigned to guanine, cytosine, and proteins (Movasaghi et al., "Raman Spectroscopy of Biological Tissues," *Appl. Spectrosc. Rev.* 42(5): 493-541 (2007), which is hereby incorporated by reference in its entirety), increased over time. This particular dataset contained markedly unusual spectra, which differed from the rest of the time points studied. The spectra were statistically considered outliers based on PCA testing.

The spectra shown in FIG. **18**, excluding those collected at 3 hours, were used to build a PLSR model. Four LVs were used, as well as Venetian blinds cross-validation. The cross-validated prediction results for the model are show in FIG. **19**. Each symbol on the plot represents a single Raman spectrum. The symbols are plotted on the x-axis according to the actual time point they were collected at, and the y-axis plots the model's cross-validated prediction for each spectrum. Ideally, the spectra would all fall on the y=x line (green trace in FIG. **19**). Instead, the line of best fit for the model deviates from this slightly (red trace in FIG. **19**). The $R^2CV$ for the PLSR model is 0.89, and the RMSECV is 0.38.

Humid Environment

After 4 days, some of the sample slides stored in the humidity chamber exhibited a fibrous gray-green covering, which appeared to be mold or mildew. Data was still collected at the 4 and 7 day time points. However, at two weeks all of the samples were completely covered in the mold-looking substance.

FIG. **20** shows the mean spectra collected in a humid environment from the first seven time points, after baseline correction, smoothing, and normalization. Spectra were not collected at 12 hours. The spectra collected from the first six hours look considerably different from the corresponding spectra collected in the ambient environment. Because of the increased humidity, it is possible that the samples were still moist upon analysis, which could affect the spectra. Additionally, the mean spectrum from 24 hours is especially void of spectral features.

The spectra shown in FIG. **20**, excluding those collected at 24 hours, were used for regression analysis. A second PLSR model was constructed using 4 LVs and Venetian blinds cross-validation. The cross-validated predictions from this model are shown in FIG. **21**. The line of best fit is slightly closer to the ideal 1:1 line, suggesting a stronger linear correlation in the spectra collected from the high humidity environment than the ambient environment. The $R^2CV$ for this PLSR model is 0.90, and the RMSECV is 0.25.

Example 10—Determination of the Age of a Saliva Stain

Raman spectroscopy was used for monitoring the changes from degradation in saliva over time. During this kinetic experiment, these biochemical changes were analyzed for eight months. The obtained results showed a great potential of Raman spectroscopy for determining the age of the saliva stain.

Saliva samples were prepared by putting a 10 μL drop on a microscopic slide, covered with aluminum foil, which reduces the fluorescence interference from glass. The following time intervals were used for this particular objective: 0 hours; 1 hour; 2 hour; 4 hours; 6 hours; 8 hours; 24 hours; 2 days; 4 days; 8 days; 14 days; 1 month; 2 months; 4 months; 8 months. Automatic mapping was performed on an area about 5×5 mm with 785 nm excitation of laser light. FIG. **22** illustrates averaged Raman spectra of saliva from the $t_0$ time point to eight months. Visual inspection of the spectroscopic changes during eight months indicated that the overall shape of the spectra, as well as positions of the bands, remain similar after one hour of body fluid degradation.

Statistical analysis was performed in MATLAB 7.4.0 software after spectral preprocessing in GRAMS/AI 7.01. The aging kinetics were studied by advanced classification methods (PLS-DA, LDA, MANOVA, SVM, ANN, etc.) with various clustering, including Mahalanobis distances-based methods, PCA, NLM, etc. In order to remove meaningless variables, feature selection methods such as t-test and P-test filters, and wrappers were used to estimate the relative importance of the independent variables in classifying the dependent variable (time). The regression model was build and cross validated as shown in FIG. **23**. As evident from these results, the regression model can be used to estimate the stain's age.

Although preferred embodiments have been depicted and described in detail herein, it will be apparent to those skilled in the relevant art that various modifications, additions, substitutions, and the like can be made without departing from the spirit of the invention and these are therefore considered to be within the scope of the invention as defined in the claims which follow.

What is claimed is:

**1**. A method of determining an age of a body fluid stain in a sample, said method comprising:

providing the sample containing a body fluid stain,

providing a statistical model for determination of the age of the body fluid stain in the sample, the statistical model based on spectroscopic signatures for a plurality modeling samples,

wherein each of the plurality of modeling samples includes a similar body fluid stain as the body fluid stain in the sample and a predetermined age of each of the body fluid stains in each modeling sample, and

wherein the spectroscopic signatures for each of the plurality of modeling samples are associated with the predetermined age of the modeling sample;

subjecting the sample or an area of the sample containing the stain to a spectroscopic analysis to produce a spectroscopic signature for the sample; and

applying the spectroscopic signature for the sample to the statistical model to ascertain the age of the body fluid stain in the sample.

**2**. The method of claim **1**, wherein the body fluid is selected from the group consisting of blood, saliva, sweat, urine, semen, and vaginal fluid.

**3**. The method of claim **1**, wherein the spectroscopic analysis is a Raman spectroscopy selected from the group consisting of resonance Raman spectroscopy, normal Raman spectroscopy, Raman microscopy, Raman microspectroscopy, NIR Raman spectroscopy, surface enhanced Raman spectroscopy (SERS), tip enhanced Raman spectroscopy